CORTICOSPINAL EXCITABILITY DURING SHORTENING AND LENGTHENING ACTIONS WITH INCREMENTAL TORQUE OUTPUT

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What is the central question of this study?

Relationship between motor unit recruitment and firing rate has been related to the size of the corticospinal output with variations in the nervous system gain during isometric contractions. However, corticospinal behaviour with incremental torque output might differ during anisometric contractions due to differences in neural control of anisometric contraction types.

What is the main finding and its importance?

Corticospinal excitability during lengthening contractions was smaller compared to shortening, but increased with incremental torque output similarly between contraction types. This suggests that the relationship between motor unit recruitment and firing rates is the likely main determinant of the size of an evoked response with variations in system gain.

ABSTRACT

The modulation of motor evoked potentials (MEPs), an index of corticospinal excitability, has been shown to increase during isometric contractions with incremental torque output in accordance with the contribution between motor unit recruitment and firing rate of the muscle to increases in required torque output. However, motor unit strategy of the muscle might not be the only factor influencing this behaviour since differences in pre- and postsynaptic control have been reported between lengthening and shortening or isometric contractions. In thirty healthy adults, MEPs were elicited in tibialis anterior during shortening and lengthening contractions at 15, 25, 50 and 80% contraction type specific maximal voluntary contraction torque. Background electromyographic activity increased progressively with greater torque output ($p<0.001$), but was similar between contraction types ($p=0.162$). When normalised to the maximal muscle response, MEPs were greater during shortening compared to lengthening

48 contractions (p=0.004) and increased step-wise with increased contraction intensities
49 (p=0.001). These data show an increase in corticospinal excitability with torque output from
50 lower to higher contraction intensities, suggesting greater contribution of motor unit
51 recruitment to increased nervous system gain in the tibialis anterior. Despite differences in
52 corticospinal control of shortening and lengthening contractions, the data suggest the
53 corticospinal responses to increases in torque output are not dependent on contraction type
54 since corticospinal excitability increased similarly during shortening and lengthening actions.
55 Thus, it is likely that the relationship between motor unit recruitment and firing rate of the
56 muscle is the main determinant of corticospinal output with variations in nervous system gain.

INTRODUCTION

The size of the motor evoked potentials (MEPs) when normalised to a maximal muscle response, an index of corticospinal excitability, is modulated by varying the nervous system gain. Data from isometric experimental models suggests that an increase in contraction strength or voluntary drive results in increased MEP size (peak to peak amplitude or area), sometimes followed by a decline, or plateau, at higher contraction strengths depending on the muscle (Goodall, Romer, & Ross, 2009; Martin, Gandevia, & Taylor, 2006; Todd, Taylor, & Gandevia, 2003; Weavil, Sidhu, Mangum, Richardson, & Amann, 2015). Specifically, muscles which rely on a greater degree of motor unit recruitment in response to an incremental force increase are likely to exhibit a peak in the evoked response at higher percentage of maximal contraction strength (Gelli, Del Santo, Popa, Mazzocchio, & Rossi, 2007; Martin et al., 2006). On the other hand, muscles that rely more on increases in motor unit firing rate for increases in force production are likely to exhibit a plateau or a decline in corticospinal excitability at lower percentages of maximal strength. This is due to the negative correlation between increases in motor unit firing rate and the probability of an evoked response (Bawa & Lemon, 1993; Brouwer, Ashby, & Midroni, 1989; Jones & Bawa, 1999). For example, the majority of arm and hand muscles (Todd *et al.*, 2003; Martin *et al.*, 2006) as well as the quadriceps (Goodall et al., 2009; Weavil et al., 2015) exhibit a peak in evoked response size followed by a decline at contraction strengths ≥ 50-75% of maximal voluntary contraction (MVC) during isometric conditions. However, the triceps brachii (Todd et al., 2003) and some lower limb muscles, such as the soleus, exhibit a continuous increase in MEP size with force output (Oya, Hoffman, & Cresswell, 2008), consistent with the relationship between motor unit recruitment and firing rates in a muscle.

The motor unit strategy of a muscle might not be the only factor determining the corticospinal output with increases in nervous system gain. For example, corticospinal excitability has been

shown to be reduced during maximal (Doguet et al., 2017; Julien Duclay, Pasquet, Martin, & Duchateau, 2011) and submaximal (Abbruzzese, Morena, Spadavecchia, & Schieppati, 1994; J. Duclay, Pasquet, Martin, & Duchateau, 2014; Gruber, Linnamo, Strojnik, Rantalainen, & Avela, 2009) lengthening compared to shortening and/or isometric contractions at a similar relative torque output. Some researchers have made inferences that the corticospinal behaviour might be similar during shortening and lengthening contractions with increases in torque output (J. Duclay et al., 2014), but these investigations have been limited in the number of contraction intensities studied. Thus, it remains contentious whether a similar trend of the contraction intensity–MEP response curve is observed during the two types of anisometric contraction. Therefore, the purpose of this study was to assess the effect of contraction intensity during shortening and lengthening contractions on the modulation of MEP amplitude. Only submaximal intensities up to 80% MVC were studied since they have a greater relevance to the activities of daily living and in order not to confound the corticospinal behaviour in the present study with potential fatigue. We hypothesised that MEPs would be lower during lengthening compared to shortening contractions, but, based on previous work in the soleus, the contraction intensity-MEP curves would exhibit similar profiles during both contractions types. The tibialis anterior (TA) was chosen as the muscle of interest due to its unique characteristics. Specifically, dorsiflexors have been shown to exhibit greater torque producing capacity during lengthening compared to shortening contraction (Pasquet *et al.*, 2000; Reeves & Narici, 2003; Klass *et al.*, 2007; Duchateau & Enoka, 2016), which might not be the case for all human muscles (Duchateau & Enoka, 2016). Furthermore, due to its role in locomotion (Byrne, O'Keeffe, Donnelly, & Lyons, 2007) and the need for accuracy of toe clearance (Capaday, Lavoie, Barbeau, Schneider, & Bonnard, 1999), the TA exhibits a facilitated corticospinal response during human walking (Capaday et al., 1999; Schubert,

106 Curt, Jensen, & Dietz, 1997) highlighting the functional need to investigate the corticospinal
107 behaviour of this specific muscle.

108

109 # METHODS

110 ## Ethical approval

111 The procedures of this study were approved by Northumbria University Faculty of Health &
112 Life Sciences Ethics Committee (RE070112538) in accordance with Declaration of Helsinki
113 with the exception of registration in a database.

114

115 ## Participants

116 Thirty healthy, young individuals (including 6 females; 25 ± 4 yrs; 175 ± 10 cm, 76.1 ± 9.9
117 kg) provided written informed consent to take part in the study. Participants were both from
118 resistance-trained (n = 4) and untrained population since previous work has shown no effect
119 of resistance-training status on MEP amplitude in the TA (Tallent, Goodall, Hortobágyi, St
120 Clair Gibson, & Howatson, 2013). A mixed-sex sample was studied to be able to extrapolate
121 the findings to a wider population. However, as highlighted recently (Sims & Heather, 2018),
122 controlling for changes in hormonal milieu is necessary for human physiological studies
123 including both sexes. To reduce the potential influence of female sex hormones on neuronal
124 function (Smith, Adams, Schmidt, Rubinow, & Wassermann, 2002), all females were tested
125 in the early follicular phase of the menstrual cycle where the quantities of both oestrogen and
126 progesterone are likely to be low (Elliott, Cable, Reilly, & Diver, 2003). The start of the early
127 follicular phase was defined as the onset of menstruation and participants were tested within 3
128 days of that. All participants were free of cardiorespiratory, neurological, neuromuscular

disorders or lower body musculoskeletal injury. They also reported no contraindications to transcranial magnetic stimulation (TMS) and were not taking any medication known to affect the nervous system. Participants refrained from caffeine and strenuous exercise for 6 and 48 hours prior to the experimental session, respectively.

Study design

Participants visited the laboratory twice, first for familiarisation followed by the experimental session 48-72 hours after. The familiarisation session included habituation with stimulation techniques and practice of the torque-matching task during different contraction types and intensities as per the experimental protocol which was performed in its entirety. During the experimental session, participants had MEPs elicited during shortening and lengthening contractions in the TA across a range of contraction intensities (15, 25, 50 and 80% of contraction type specific MVC). The order of contraction type and intensity were randomised. Eight trials were performed per contraction type and intensity and those responses were averaged and used for data analysis. The experimental protocol is depicted in Figure 1.

Procedures

Experimental setup

Participants were sat in an isokinetic dynamometer (Cybex Norm, NY, USA) with the foot of the dominant limb strapped firmly on the motor plate of the device with the knee and hip kept at 120° (180□ = full extension) and 90°, respectively. Contractions were performed by assisting or resisting the power head of the device during shortening and lengthening actions, respectively, as the ankle moved through a 30° range of motion (75° to 105°) at an angular

velocity of 15°·s^{-1}. All stimuli were applied as the ankle passed through anatomical zero (90°). Prior to eliciting MEPs during shortening and lengthening dorsiflexion, a specific contraction type MVC was performed (Škarabot et al., 2018), with the MVC torque value recorded at anatomical zero.

Electromyography

Surface electromyography (EMG) was recorded using bipolar EMG electrodes (8 mm diameter, 20 mm inter-electrode distance; Kendall 1041PTS, Tyco Healthcare Group, MA, USA) placed on the belly of TA at one-third of the length between the head of the fibula and the medial malleolus with the reference electrode on the medial malleolus according to SENIAM recommendations (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). Prior to placement of electrodes, the recording site was shaved, abraded with preparation gel and wiped clean with an alcohol swab to ensure appropriate impedance (< 2 kΩ). The EMG signal was amplified (×1000), band pass filtered (10-1000 Hz; D360, Digitimer, Hertfordshire, UK) and sampled at 5 kHz (CED Power 1401, Cambridge Electronic Design, UK).

Transcranial magnetic stimulation

A magnetic stimulator (Magstim 200^2, Magstim Ltd., UK; maximal output of ~1.4 T) with a posterior-to-anterior current, 110 mm double-cone coil was used to evoke MEPs in TA of the dominant leg. Initially, the coil was positioned over the reported optimal spot for stimulation of the TA muscle, roughly 0.5-1 cm lateral and posterior to the vertex (Devanne, Lavoie, & Capaday, 1997), after which the coil was moved around the initial spot in small steps until the position evoking the biggest potential in TA (hotspot) was found. After that, resting motor

threshold (RMT) was determined, defined as a stimulus intensity that evoked peak-to-peak MEP amplitude ≥ 50 μV in 5 out of 10 trials (Rossini et al., 2015). During the experiment, the pulses were delivered at 1.2 × RMT as it lies on the middle portion of the ascending part of the stimulus-response curve (Han, Kim, & Lim, 2001) and is thus sensitive to changes in corticospinal excitability. All contractions were separated at least 30 seconds to ensure MEPs had returned to resting values (Tallent et al., 2012).

Percutaneous nerve stimulation

The maximal muscle response (M_{max}) was elicited with a 40 mm diameter cathode/anode arrangement over the peroneal nerve (1 ms pulse duration; Digitimer DS7AH, Welwyn Garden City, Hertfordshire, UK). Once optimal electrode location had been identified, it was marked with a permanent marker and the electrode was strapped to participant's leg. The current of the stimulation was increased until no further increase in the evoked response during rest was observed, after which the current was additionally increased by 50% to ensure supramaximal intensity of stimulation. To account for possibility of M_{max} modulation with contraction type and intensity (Lee & Carroll, 2005), M_{max} was then elicited during specific muscle action (shortening and lengthening) and intensity (15, 25, 50 and 80% of contraction type specific MVC) and later used to normalise responses to TMS.

Data analysis

All data were recorded in a 500 ms window including 50 ms before TMS was delivered (Signal v3, CED, UK). Background EMG activity was assessed as the mean rectified EMG activity obtained 25 ms prior to the stimulus and was normalised to peak-to-peak amplitude of

198 M_{max} (EMG/M_{max}; Lanza *et al.*, 2018). Peak-to-peak amplitude of MEP was calculated and
199 expressed relative to the amplitude of M_{max} (MEP/M_{max}).

200

201 **Statistical analysis**

202 A 2 × 4 ANOVA (2 – contraction type, 4 – contraction intensity) was performed to assess the
203 effect of contraction type and intensity on EMG/M_{max} and MEP/M_{max} (SPSS Inc., Chicago,
204 IL, USA). Post hoc analyses were performed using pairwise comparison with Bonferroni
205 correction. Significance was accepted at an alpha level of 0.05. All data are presented as
206 means ± standard deviation (SD).

207

208 **RESULTS**

209 EMG/M_{max} increased with contraction intensity ($F_{1.2, 33.4} = 101.8$, $p < 0.001$, $\eta_p^2 = 0.78$) in a
210 progressive manner ($p < 0.001$ for all; Figure 2A), but was similar between contraction types
211 ($F_{1, 29} = 2.0$, $p = 0.171$, $\eta_p^2 = 0.06$). However, MEP/M_{max} was greater during shortening
212 compared to lengthening contractions ($F_{1, 29} = 13.6$, $p = 0.001$, $\eta_p^2 = 0.32$), but also increased
213 with contraction intensity ($F_{1.6, 46.6} = 86.6$, $p < 0.001$, $\eta_p^2 = 0.75$) in a step-wise manner ($p <$
214 0.001 for all; Figure 2B) during both contraction types.

215 The continual increase in corticospinal excitability across contraction intensities is also shown
216 in a representative example (Figure 3). MEP/M_{max} ratio in this participant increased from 0.38
217 to 0.42, 0.46 and 0.56 during shortening and from 0.30 to 0.38, 0.40 and 0.50 during
218 lengthening contractions at 15, 25, 50 and 80% MVC. The behaviour was similar across all
219 participants (Figure 4).

DISCUSSION

The present data showed an increase in the size of the MEP amplitude with incremental torque output up to 80% of maximal torque regardless of contraction type. This is the first study to have explored the behaviour of corticospinal excitability with varying nervous system gain and corroborates the experiments using isometric experimental models on other lower leg muscles such as gastrocnemius and soleus where continual increase in corticospinal and spinal excitability was observed with increments in torque output (Oya et al., 2008). This has been related to the motor unit recruitment being the primary strategy for incremental force control in plantarflexors (Grillner & Udo, 1971). However, the opposite is seen in muscles such as brachioradialis where increases in firing rate largely contribute to increments in force production at forces ≥ 75% MVC, resulting in a decrease in corticospinal excitability at high contraction strengths (Martin et al., 2006). Specifically, this decrease in corticospinal excitability has been related to unresponsiveness of the motoneuron pool to an external stimulus, which is likely due to refractory motoneurons as part of the after-hyperpolarisation of an action potential (Martin et al., 2006). As evidenced by animal work (Baldissera & Gustafsson, 1974; Schwindt & Calvin, 1972) and modelling studies (Jones & Bawa, 1999; Matthews, 1999), motoneurons exhibit an exponential return to threshold when firing rates are lower, whereas a more progressive return is evident with a greater discharge rate. Data from single motor unit recordings suggests that during non-ballistic contractions the TA exhibits a progressive recruitment of motor units up to ~90% MVC (Desmedt & Godaux, 1977; Van Cutsem, Duchateau, & Hainaut, 1998). Thus, given motor unit recruitment is the primary strategy of the TA for increasing force output, the graded increase in corticospinal excitability across contraction intensities in the present study supports the notion that the behaviour of evoked potentials is related to the motor unit strategy of a muscle.

Another factor that can affect the behaviour of corticospinal excitability with variations in torque output is the TMS stimulus intensity. It has been shown in upper limb muscles, with lower a stimulus intensity, a peak in MEP, followed by a MEP decline, occurs at a greater percentage of MVC compared to higher stimulus intensities (Martin et al., 2006). However, in lower limb muscles with smaller firing frequency of motor units, the decline in MEP at higher contraction intensity was not observed with higher stimulus intensity; rather the responses only plateaued (Oya et al., 2008). We did not examine the responses at intensities corresponding to maximal MEP amplitude and thus this question could be an avenue worth exploring in future investigations since it remains possible that different results would be obtained in the present study had greater TMS stimulus intensity been used. Furthermore, it has been suggested that the weaker projections to lower limb muscles might be responsible for the continual increase in corticospinal excitability with increased contraction strengths (Oya et al., 2008). However, the present data in the TA, a muscle which has a preferential input from the pyramidal tract into the spinal networks (Brooks & Stoney, 1971), and similar strength of corticomotoneuronal projections to the upper limbs (Brouwer & Ashby, 1990), suggest this is not the case. Therefore, it appears that motor unit control is still the primary factor determining a corticospinal response as the contraction intensity increases.

The continual increase of corticospinal output was observed during both shortening and lengthening contractions, despite a reduced corticospinal excitability during lengthening relative to shortening contractions, in line with other muscles of the upper and lower limbs (J. Duclay et al., 2014; Gruber et al., 2009). A previous study has shown a continual increase in corticospinal excitability from submaximal to maximal contraction strength in the soleus (J. Duclay et al., 2014), but the study was limited in the contraction strengths studied (only 50% and 100% MVC). Accordingly, the present study extends this observation of corticospinal excitability modulation with variations in torque output regardless of contraction type, across

differing submaximal intensities. Unlike the soleus, the difference in corticospinal output between shortening and lengthening contractions in medial gastrocnemius during submaximal contractions was absent during an MVC (J. Duclay et al., 2014). Since the present study did not investigate corticospinal excitability during maximal shortening and lengthening actions due to their lack of relevance in functional activities of daily living, it remains unknown whether similar behaviour is evident in TA. Thus, it is important to consider the responses observed in the present study in the context of the muscle and contraction intensities investigated. Whilst maximal contraction intensities are rarely going to be relevant for activities of daily living, inclusion of responses to TMS during maximal contractions, that represent the limit of torque producing capacity of an individual, and muscles with different motor unit recruitment strategies (e.g. the TA versus a hand muscle) would provide a more complete picture of corticospinal behaviour during different muscle actions with incremental torque output and might be something worth exploring in future studies. Given the reported motor unit control strategy of the TA with incremental torque output, it could be hypothesised that the evoked responses would peak at ~90% MVC (Desmedt & Godaux, 1977; Van Cutsem et al., 1998).

In conclusion, the contraction type–response curve of MEPs is similar during shortening and lengthening contractions when normalised to a contraction specific MVC. Despite differences in neural control of shortening and lengthening contractions, it appears that the relationship between motor unit recruitment and firing rate is the main mechanism determining corticospinal output with variations in nervous system gain.

REFERENCES

Abbruzzese, G., Morena, M., Spadavecchia, L., & Schieppati, M. (1994). Response of arm flexor muscles to magnetic and electrical brain stimulation during shortening and lengthening tasks in man. *The Journal of Physiology*, *481*, 499–507. http://doi.org/10.1113/jphysiol.1994.sp020458

Baldissera, F., & Gustafsson, B. (1974). Firing behaviour of a neurone model based on the afterhyperpolarization conductance time course. First interval firing. *Acta Physiologica Scandinavica*, *91*(4), 528–544. http://doi.org/10.1111/j.1748-1716.1974.tb05708.x

Bawa, P., & Lemon, R. N. (1993). Recruitment of motor units in response to transcranial magnetic stimulation in man. *The Journal of Physiology*, *471*, 445–464. http://doi.org/10.1111/j.1748-1716.1974.tb05708.x

Brooks, V. B., & Stoney, S. D. (1971). Motor Mechanisms: The Role of the Pyramidal System in Motor Control. *Annual Review of Physiology*, *33*(1), 337–388. http://doi.org/10.1146/annurev.ph.33.030171.002005

Brouwer, B., & Ashby, P. (1990). Corticospinal projections to upper and lower limb spinal motoneurons in man. *Electroencephalography and Clinical Neurophysiology*, *76*(6), 509–519. http://doi.org/10.1016/0013-4694(90)900002-2

Brouwer, B., Ashby, P., & Midroni, G. (1989). Excitability of corticospinal neurons during tonic muscle contractions in man. *Experimental Brain Research*, *74*(3), 649–652. http://doi.org/10.1007/BF00247369

Byrne, C. A., O'Keeffe, D. T., Donnelly, A. E., & Lyons, G. M. (2007). Effect of walking speed changes on tibialis anterior EMG during healthy gait for FES envelope design in drop foot correction. *Journal of Electromyography and Kinesiology*, *17*(5), 605–616.

http://doi.org/10.1016/j.jelekin.2006.07.008

Capaday, C., Lavoie, B. A., Barbeau, H., Schneider, C., & Bonnard, M. (1999). Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. *Journal of Neurophysiology*, *81*(1), 129–139. http://doi.org/10.1152/jn.1999.81.1.129

Desmedt, J. E., & Godaux, E. (1977). Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. *The Journal of Physiology*, *264*(3), 673–693. http://doi.org/10.1113/jphysiol.1977.sp011689

Devanne, H., Lavoie, B. A., & Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Experimental Brain Research*, *114*(2), 329–338. http://doi.org/10.1007/PL00005641

Doguet, V., Nosaka, K., Guével, A., Thickbroom, G., Ishimura, K., & Jubeau, M. (2017). Muscle length effect on corticospinal excitability during maximal concentric, isometric and eccentric contractions of the knee extensors. *Experimental Physiology*, *102*, 1513–1523. http://doi.org/10.1113/EP086480

Duchateau, J., & Enoka, R. M. (2016). Neural control of lengthening contractions. *The Journal of Experimental Biology*, *219*(Pt 2), 197–204. http://doi.org/10.1242/jeb.123158

Duclay, J., Pasquet, B., Martin, A., & Duchateau, J. (2011). Specific modulation of corticospinal and spinal excitabilities during maximal voluntary isometric, shortening and lengthening contractions in synergist muscles. *The Journal of Physiology*, *589*(Pt 11), 2901–2916. http://doi.org/10.1113/jphysiol.2011.207472

Duclay, J., Pasquet, B., Martin, A., & Duchateau, J. (2014). Specific modulation of spinal and cortical excitabilities during lengthening and shortening submaximal and maximal

337 contractions in plantar flexor muscles. *Journal of Applied Physiology*, *117*(12), 1440–
338 1450. http://doi.org/10.1152/japplphysiol.00489.2014

339 Elliott, K., Cable, N., Reilly, T., & Diver, M. (2003). Effect of menstrual cycle phase on the
340 concentration of bioavailable 17-β oestradiol and testosterone and muscle strength.
341 *Clinical Science*, *105*(6), 663–669. http://doi.org/10.1042/CS20020360

342 Gelli, F., Del Santo, F., Popa, T., Mazzocchio, R., & Rossi, A. (2007). Factors influencing the
343 relation between corticospinal output and muscle force during voluntary contractions.
344 *The European Journal of Neuroscience*, *25*(11), 3469–3475.
345 http://doi.org/10.1111/j.1460-9568.2007.05590.x

346 Goodall, S., Romer, L. M., & Ross, E. Z. (2009). Voluntary activation of human knee
347 extensors measured using transcranial magnetic stimulation. *Experimental Physiology*,
348 *94*(9), 995–1004. http://doi.org/10.1113/expphysiol.2009.047902

349 Grillner, S., & Udo, M. (1971). Recruitment in the tonic stretch reflex. *Acta Physiologica*
350 *Scandinavica*, *81*(4), 571–573. http://doi.org/10.1111/j.1748-1716.1971.tb04935.x

351 Gruber, M., Linnamo, V., Strojnik, V., Rantalainen, T., & Avela, J. (2009). Excitability at the
352 motoneuron pool and motor cortex is specifically modulated in lengthening compared to
353 isometric contractions. *Journal of Neurophysiology*, *101*(4), 2030–2040.
354 http://doi.org/10.1152/jn.91104.2008

355 Han, T. R., Kim, J. H., & Lim, J. Y. (2001). Optimization of facilitation related to threshold in
356 transcranial magnetic stimulation. *Clinical Neurophysiology : Official Journal of the*
357 *International Federation of Clinical Neurophysiology*, *112*(4), 593–599.
358 http://doi.org/10.1016/S1388-2457(01)00471-0

359 Hermens, H. J., Freriks, B., Disselhorst-Klug, C., & Rau, G. (2000). Development of

recommendations for SEMG sensors and sensor placement procedures. *Journal of Electromyography and Kinesiology*, *10*(5), 361–374. http://doi.org/10.1016/S1050-6411(00)00027-4.

Jones, K. E., & Bawa, P. (1999). A comparison of human motoneuron data to simulated data using cat motoneuron models. *Journal of Physiology, Paris*, *93*(1–2), 43–59. http://doi.org/10.1016/S0928-4257(99)80135-1

Klass, M., Baudry, S., & Duchateau, J. (2007). Voluntary activation during maximal contraction with advancing age: a brief review. *European Journal of Applied Physiology*, *100*(5), 543–551. http://doi.org/10.1007/s00421-006-0205-x

Lanza, M. B., Balshaw, T. G., Massey, G. J., & Folland, J. P. (2018). Does normalization of voluntary EMG amplitude to MMAX account for the influence of electrode location and adiposity? *Scandinavian Journal of Medicine & Science in Sports*. http://doi.org/10.1111/sms.13270

Lee, M., & Carroll, T. J. (2005). The amplitude of Mmax in human wrist flexors varies during different muscle contractions despite constant posture. *Journal of Neuroscience Methods*, *149*(2), 95–100. http://doi.org/10.1016/j.jneumeth.2005.03.016

Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2006). Output of human motoneuron pools to corticospinal inputs during voluntary contractions. *Journal of Neurophysiology*, *95*(6), 3512–8. http://doi.org/10.1152/jn.01230.2005

Matthews, P. (1999). The effect of firing on the excitability of a model motoneurone and its implications for cortical stimulation. *The Journal of Physiology*, *518*(3), 867–882. http://doi.org/10.1111/j.1469-7793.1999.0867p.x

Oya, T., Hoffman, B., & Cresswell, A. (2008). Corticospinal-evoked responses in lower limb

muscles during voluntary contractions at varying strengths. *Journal of Applied Physiology (1985)*, *115*(5), 1527–1532. http://doi.org/10.1152/japplphysiol.90586.2008

Pasquet, B., Carpentier, A., Duchateau, J., & Hainaut, K. (2000). Muscle fatigue during concentric and eccentric contractions. *Muscle & Nerve*, *23*(11), 1727–1735. http://doi.org/10.1002/1097-4598(200011)23:11<1727::AID-MUS9>3.0.CO;2-Y

Reeves, N. D., & Narici, M. V. (2003). Behavior of human muscle fascicles during shortening and lengthening contractions in vivo. *Journal of Applied Physiology*, *95*(3), 1090–1096. http://doi.org/10.1152/japplphysiol.01046.2002

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., … Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*, *126*(6), 1071–1107. http://doi.org/10.1016/j.clinph.2015.02.001

Schubert, M., Curt, A., Jensen, L., & Dietz, V. (1997). Corticospinal input in human gait: modulation of magnetically evoked motor responses. *Experimental Brain Research*, *115*(2), 234–246. http://doi.org/10.1007/PL00005693

Schwindt, P. C., & Calvin, W. H. (1972). Membrane-potential trajectories between spikes underlying motoneuron firing rates. *Journal of Neurophysiology*, *35*(3), 311–325. http://doi.org/10.1152/jn.1972.35.3.311

Sims, S. T., & Heather, A. K. (2018). Myths and Methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. *Experimental Physiology*. http://doi.org/10.1113/EP086797

Škarabot, J., Ansdell, P., Brownstein, C., Howatson, G., Goodall, S., & Durbaba, R. (2018).

406 Differences in force normalising procedures during submaximal anisometric
407 contractions. *Journal of Electromyography and Kinesiology*, *41*, 82–88.
408 http://doi.org/10.1016/j.jelekin.2018.05.009

409 Smith, M. J., Adams, L. F., Schmidt, P. J., Rubinow, D. R., & Wassermann, E. M. (2002).
410 Effects of ovarian hormones on human cortical excitability. *Annals of Neurology*, *51*(5),
411 599–603. http://doi.org/10.1002/ana.10180

412 Tallent, J., Goodall, S., Hortobágyi, T., Clair Gibson, A. S., French, D. N., & Howatson, G.
413 (2012). Recovery time of motor evoked potentials following lengthening and shortening
414 muscle action in the tibialis anterior. *Journal of Clinical Neuroscience*, *19*(9), 1328–
415 1329. http://doi.org/10.1016/j.jocn.2012.01.022

416 Tallent, J., Goodall, S., Hortobágyi, T., St Clair Gibson, A., & Howatson, G. (2013).
417 Corticospinal responses of resistance-trained and un-trained males during dynamic
418 muscle contractions. *Journal of Electromyography and Kinesiology*, *23*(5), 1075–1081.
419 http://doi.org/10.1016/j.jelekin.2013.04.014

420 Todd, G., Taylor, J. L., & Gandevia, S. C. (2003). Measurement of voluntary activation of
421 fresh and fatigued human muscles using transcranial magnetic stimulation. *The Journal*
422 *of Physiology*, *551*(2), 661–671. http://doi.org/10.1113/jphysiol.2003.044099

423 Van Cutsem, M., Duchateau, J., & Hainaut, K. (1998). Changes in single motor unit
424 behaviour contribute to the increase in contraction speed after dynamic training in
425 humans. *The Journal of Physiology*, *513 (Pt 1)*, 295–305. http://doi.org/10.1111/j.1469-
426 7793.1998.295by.x

427 Weavil, J., Sidhu, S., Mangum, T., Richardson, R., & Amann, M. (2015). Intensity-dependent
428 alterations in the excitability of cortical and spinal projections to the knee extensors

during isometric and locomotor exercise. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *308*(12), R998-1007. http://doi.org/10.1152/ajpregu.00021.2015

Competing interests

The authors have no competing interests to declare, financial or otherwise.

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Author Contributions

Experiments were performed in the Biomechanics Laboratory at Northumbria University. JŠ, JT, SG and GH designed the study protocol; JT acquired the data; JŠ, JT, SG, RD and GH analysed and interpreted the data; JŠ, JT, SG, RD and GH drafted or revised the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons listed qualify for authorship.

449 **Figure captions**

450 Figure 1. The experimental protocol. Participants first performed maximal shortening and
451 lengthening contractions (MVC; randomised order) which was subsequently used for
452 calculation of submaximal contraction intensities. Thereafter, participants performed
453 shortening and lengthening contractions at 15, 25, 50 and 80% of contraction type
454 specific MVC (pseudorandomised order) whilst receiving transcranial magnetic
455 stimulation (black downward arrow) and percutaneous stimulation over the peroneal
456 nerve (grey downward arrow) to elicit motor evoked potentials (MEP) and maximal
457 compound action potentials (M_{max}), respectively. Participants performed the same
458 protocol 2-3 days prior to the experimental session for the purposes of familiarisation.

459 Figure 2. Grouped data (mean ± SD) for background EMG activity (EMG/M_{max}; A) and
460 motor evoked potentials normalised to maximal muscle response (MEP/M_{max}; B) during
461 shortening and lengthening contractions at 15, 25, 50 and 80% of contraction-type
462 specific maximal torque. $^{*}p < 0.005$ relative to lengthening, $^{\#}p < 0.001$ relative to other
463 contraction strengths.

464 Figure 3. A representative example of motor evoked potentials across contraction intensities
465 (15, 25, 50 and 80% MVC torque) during shortening (A) and lengthening (B)
466 contractions from an individual that best represents the sample mean. Responses are
467 shown from 50 ms before to 250 ms after the stimulus (as denoted by the vertical line
468 representing the stimulus artefact). Each trace is an average of 8 waveforms.

469 Figure 4. Individual data for motor evoked potentials in tibialis anterior normalised to
470 maximal muscle response during shortening and lengthening contractions at 15, 25, 50
471 and 80% of contraction-type specific maximal torque.

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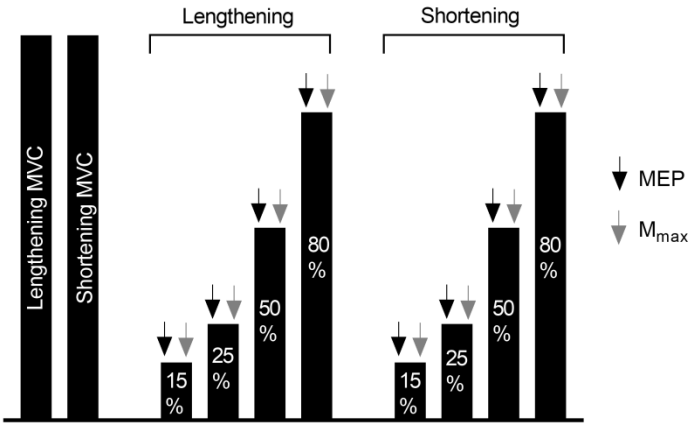
473 Figure 1



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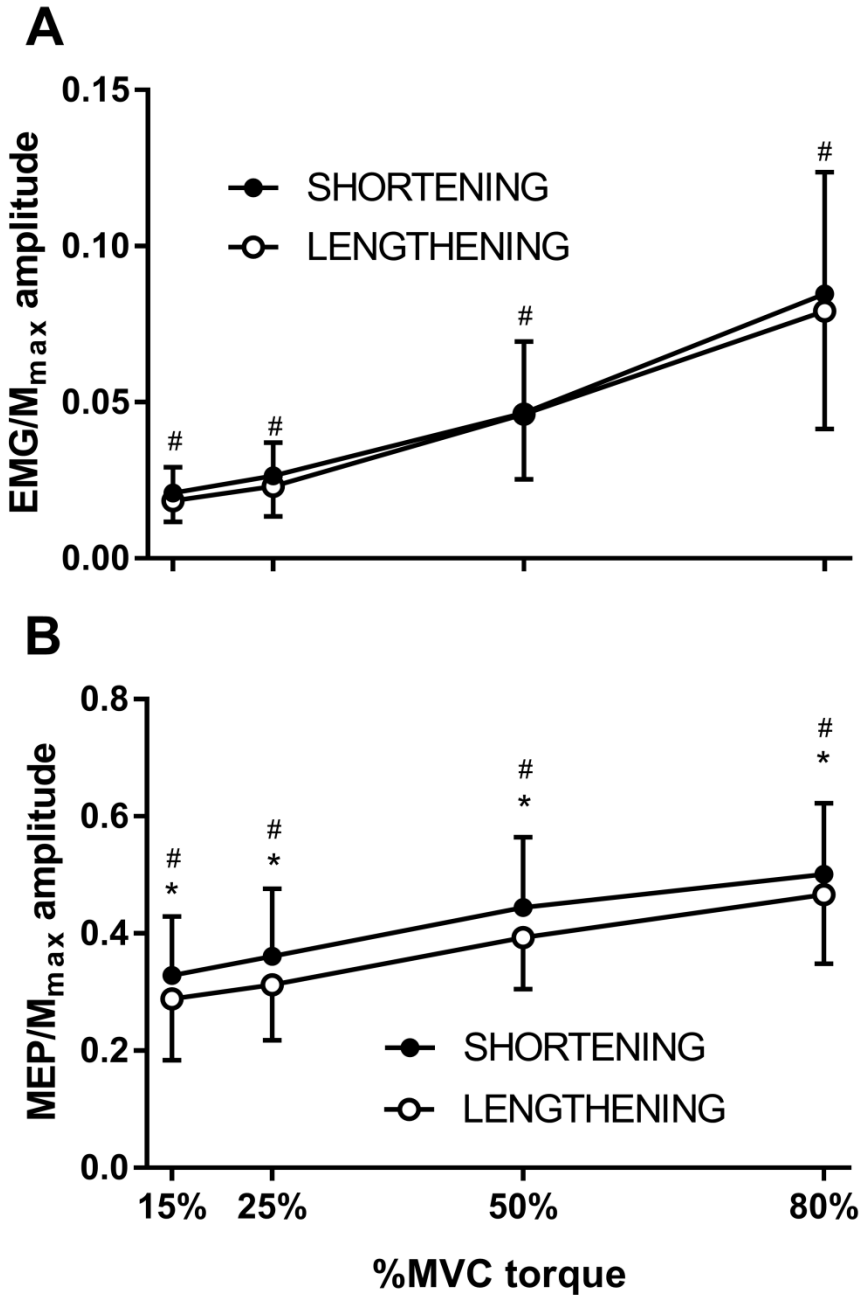
476 Figure 2



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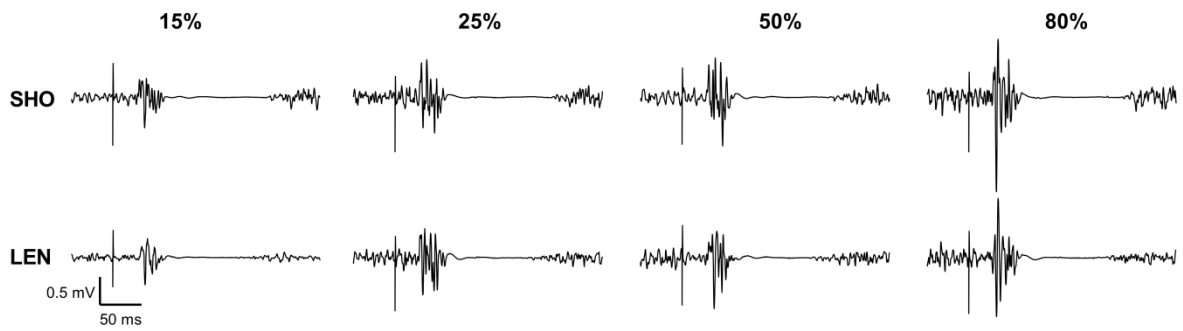
479 Figure 3



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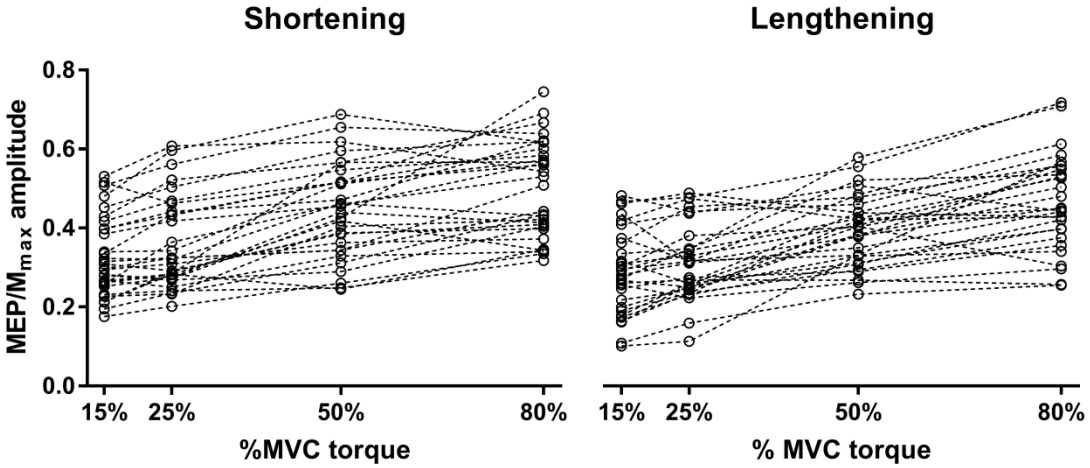
482 Figure 4



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